INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02874

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07K 7/06,7/08,15/28; G01N 33/543,569,571,577

U.S.Cl.: 435/5, 7, 810; 436/570, 578,548; 530/327,328,387,806,809; 935/110

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/5,7,810; 436/510,518,548; 530/327,328,387, 806,809; 935/110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

BIOSIS searches: 1) Monoclonal (10A) (ARV OR HIV or LAV or HIV) and (P24 or P26);
2) (PEPTIDE # or OLIGOPEPTIDE #) and (ARV or HTLV or LAV

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
P,X	US, A, 4,843,011 (SARNGADHARAN et al.) 27 June 1989. See col. 5, lines 35-56 2nd col. 6, lines 38-60.	1-7
P,X	US, A, 4,888,290 (KORTRIGHT et al.) 19 December 1989. See col. 2, lines 55-64 and col. 4, line 42-col. 5, line 5.	1-7, 12, 17-19, 24 and 25
X	Biological Abstracts, vol. 86, No. 10, issued 15 November 1988, M. Niedrig et al., "Monoclonal antibodies directed against human immunodeficiency virus (HIV) gag proteins with specificity for conserved epitopes in HIV-2 and simian immunodeficiency virus". See page 1270, col. 1, the abstract no. 109338.	1-7
P,X,	Biological Abstracts, vol. 87, no. 10, issued 15 May 1989, P, Kusk et al., "Immunological characterization and detection of the major core protein p24 of the human immunodeficiency virus (HIV) using monoclonal antibodies". See page 1146, col. 1, the abstract no. 110752.	1-7, and 12-25.

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [1]
15 August 1990	04SEP 1990
International Searching Authority [1]	**Signature of Authorized Officer** [2]
ISA/US	NGUYEN NGOC-HO INTERNATIONAL DIVISION David A. Saunders

FURTHER INFORMATION CONTINUED FR M THE SEC ND SHEET

X	Biological Abstracts, Vol. 84, no. 1, issued 01 July 1987, T. Hattori et al., "Characteracterization of three monoclonal antibodies (VAK3-5) that identify p24, core protein of human immunodeficiency virus and its precursors". See page 499, col. 1, the abstract no. 445.9	1-7
X	Biological Abstracts, vol. 80, no. 10, issued 15 November 1985, V. DI Marzo et al., "Monoclonal antibodies specific for p24, the major core protein of human T cell leukemia virus type III". See page 587, col. 2, the abstract no. 87756.	1-7

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers .. because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

continued from I. Classification of subject matter block 3.

or HIV) and (P24 or P26).

III. DOCUMENTS CONSIDERED TO BE RELEVANT (C NTINUED FR M THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
X	US, A, 4,755,457 ROBERT-GUROFF et al.) 05 July 1988. See col. 2, lines 27-29.	1-7

FIG. 1

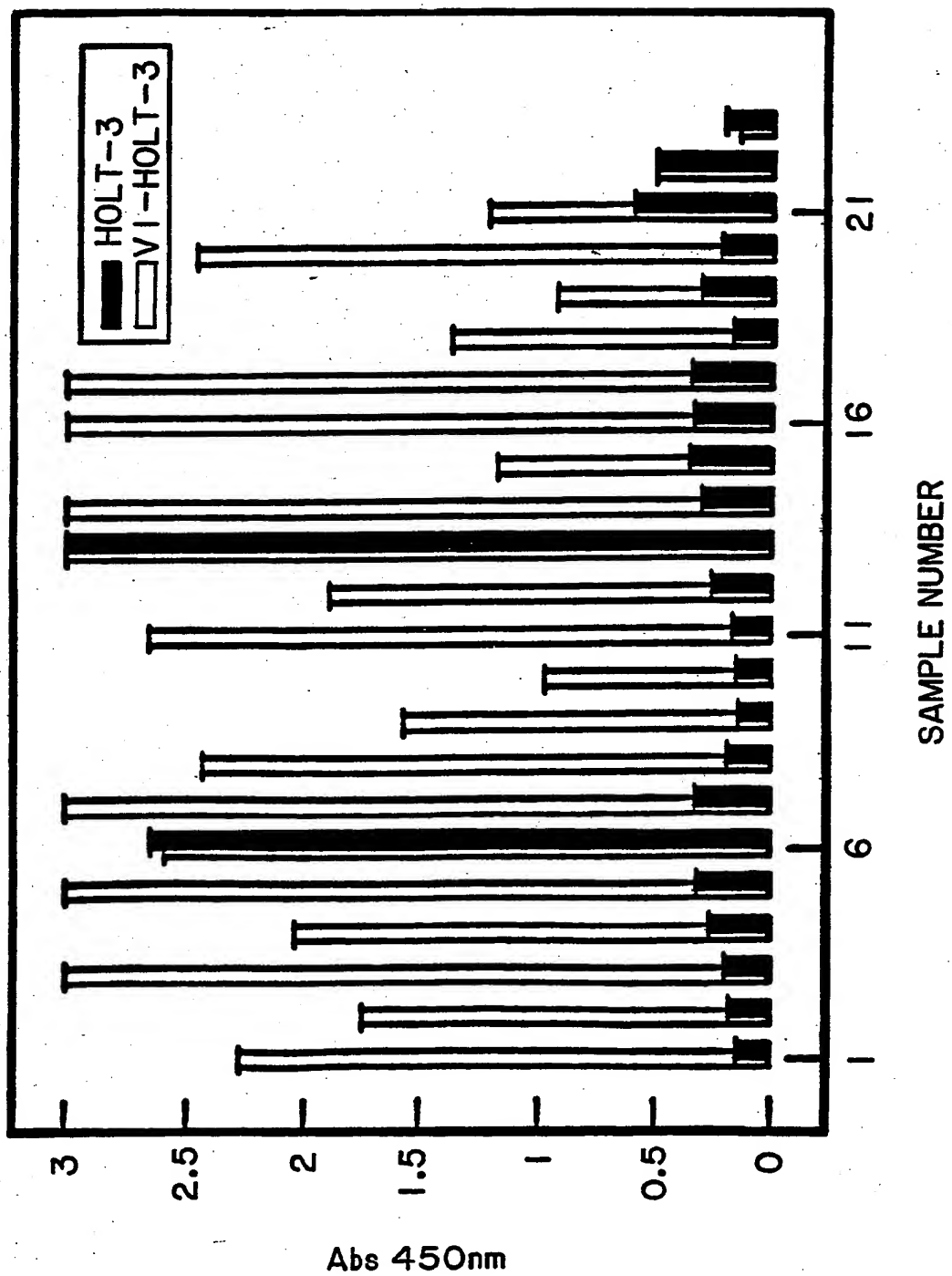

HOLT-3
VI-HOLT-3
3
2.5
2
1.5
1
0.5
0
Abs 450nm
1
6
11
16
21
SAMPLE NUMBER

FIG. 2a

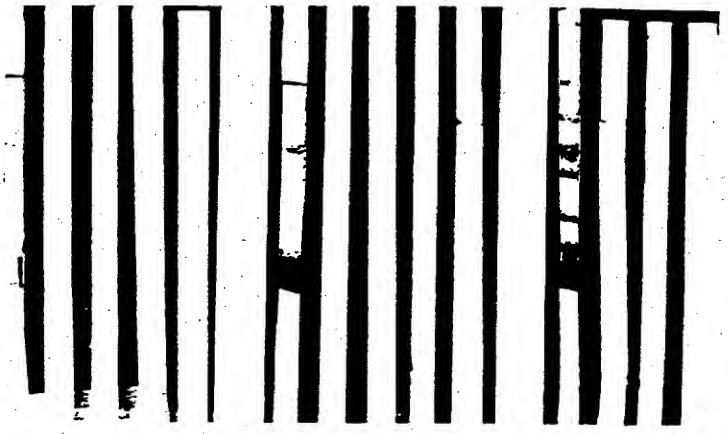


FIG. 2b

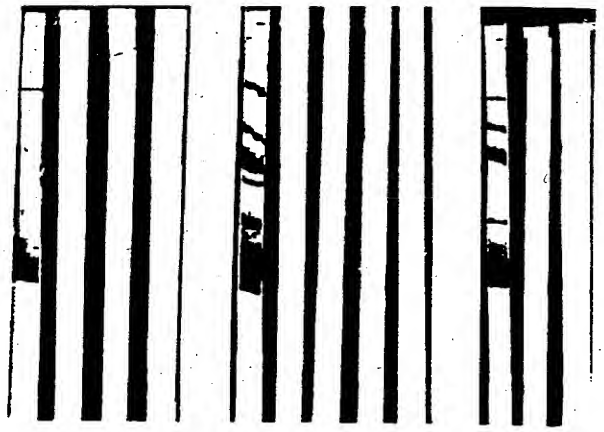
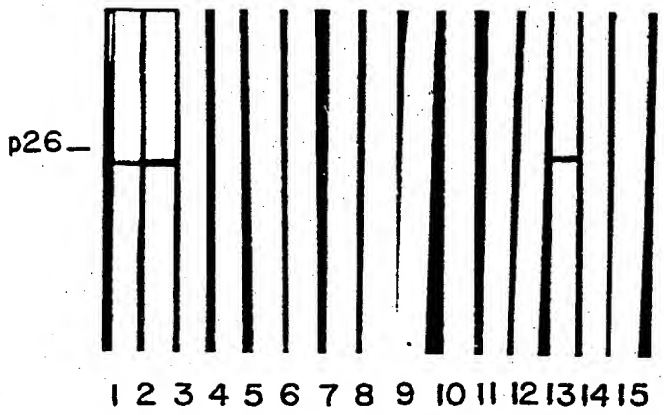


FIG. 3



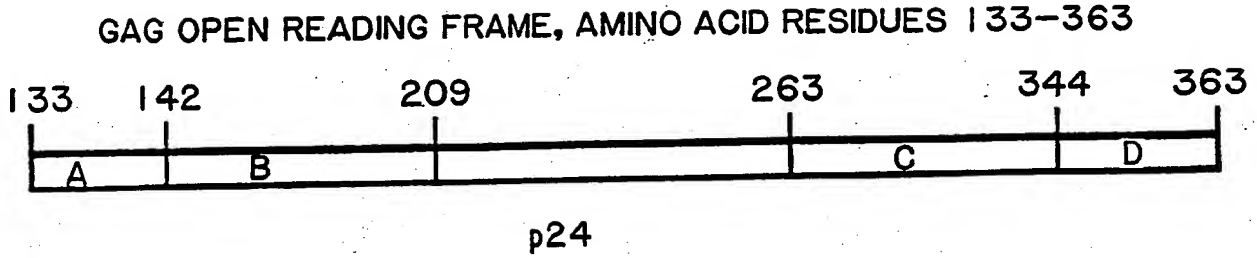

GAG OPEN READING FRAME, AMINO ACID RESIDUES 133–363
133
142
209
263
344
363
A
B
C
D
p24

FIG. 5

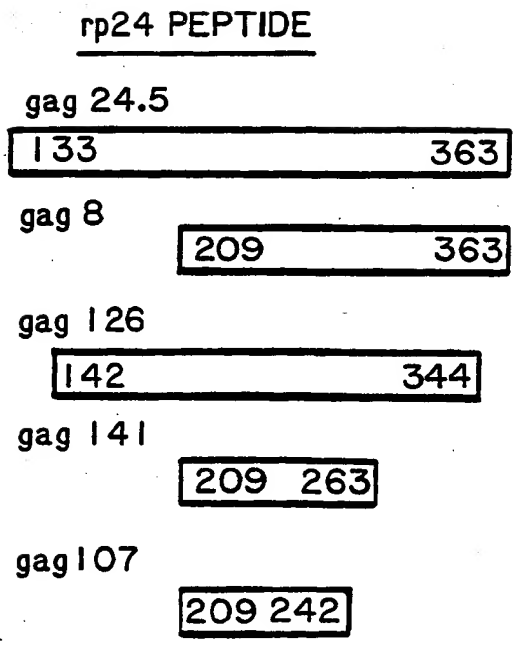

rp24 PEPTIDE
gag 24.5
133
363
gag 8
209
363
gag 126
142
344
gag 141
209 263
gag 107
209 242

FIG. 4

FIG. 6a

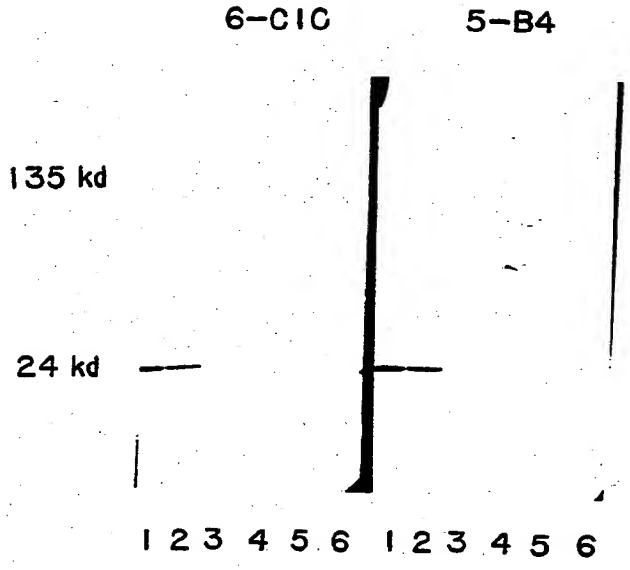

6-C1C
5-B4
135 kd
24 kd
1 2 3 4 5 6 1 2 3 4 5 6

FIG. 6b

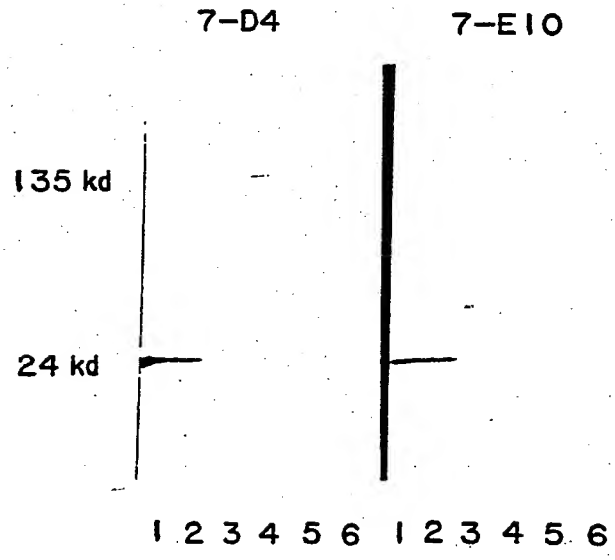

7-D4
7-E10
135 kd
24 kd
1 2 3 4 5 6 1 2 3 4 5 6

FIG. 7

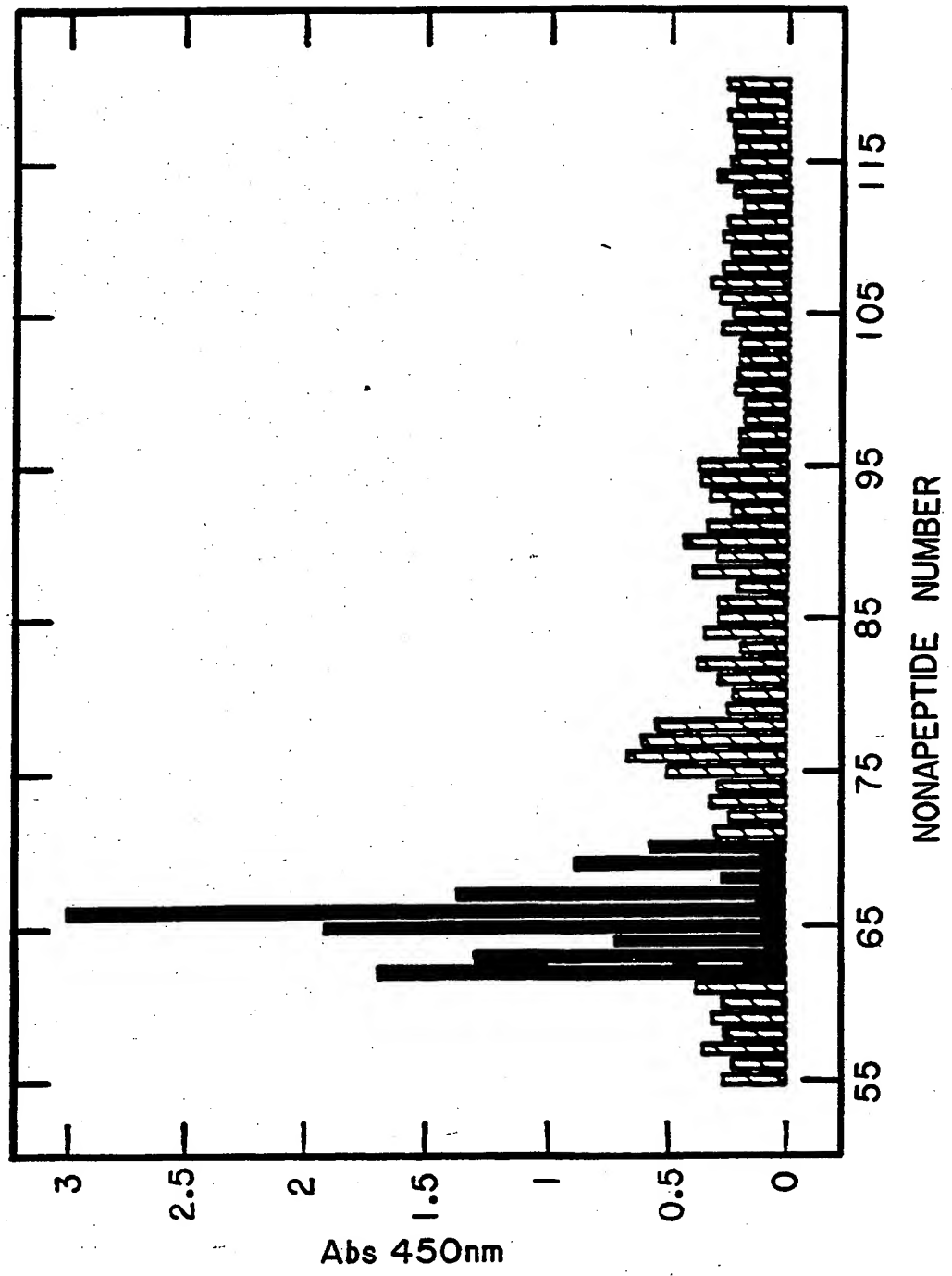

NONAPEPTIDE NUMBER
55
65
75
85
95
105
115
Abs 450nm
0
0.5
1
1.5
2
2.5
3

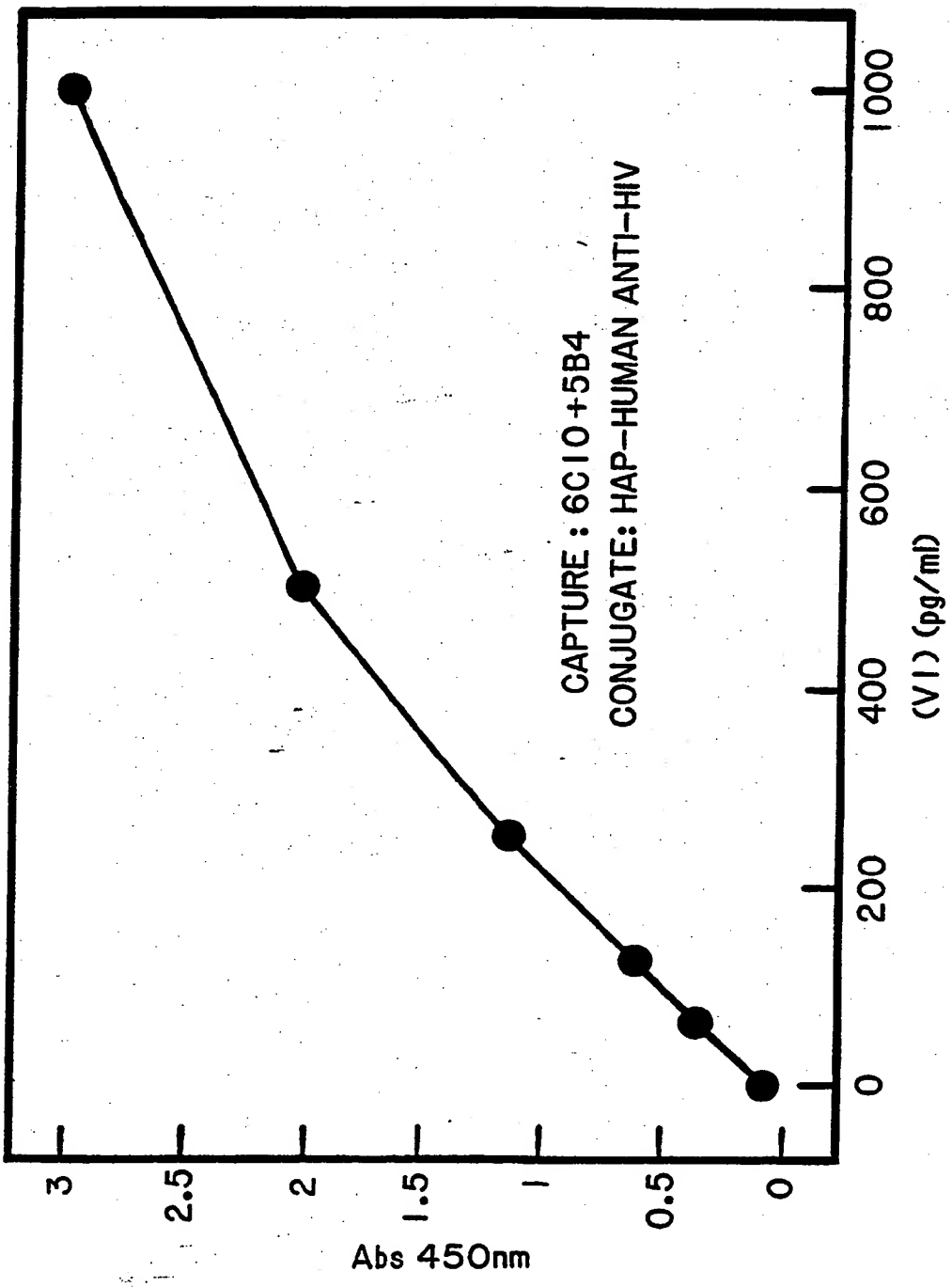
FIG. 8

NONAPEPTIDE FRACTION	AMINO ACID SEQUENCE	ELISA
61	Gln-Met-Val-His-Gln-Ala-Ile-Ser-Pro	-
62	Met-Val-His-Gln-Ala-Ile-Ser-Pro-Arg	+
63	Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr	+
64	His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu	+
65	Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn	+
66	Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala	+
67	Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp	+
68	Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val	-
69	Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys	+
70	Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-Val	+
71	Thr-Leu-Asn-Ala-Trp-Val-Lys-Val-Val	-
	COMPOSITE (amino acid residues 142-158):	
	HIV-1_{RF}: -Gln-Met-Val-[His]-Gln-Ala-Ile-[Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys]-[Val]-[Val] (142, 158)	+

FIG. 9

	142 ... 158	
HIV-1_{RF}:	-Gln-Met-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-Val-Val-	+
HIV-2_{NIH-Z}:	-Asn-Tyr-Thr-His-Ile-Pro-Leu-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-Leu-Val-	+
SIV_{MAC}:	-Asn-Tyr-Thr-His-Leu-Pro-Leu-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-Leu-Val-	+

FIG. 10


CAPTURE : 6C10+5B4
CONJUGATE: HAP-HUMAN ANTI-HIV
Abs 450nm
3
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
(V1) (pg/ml)

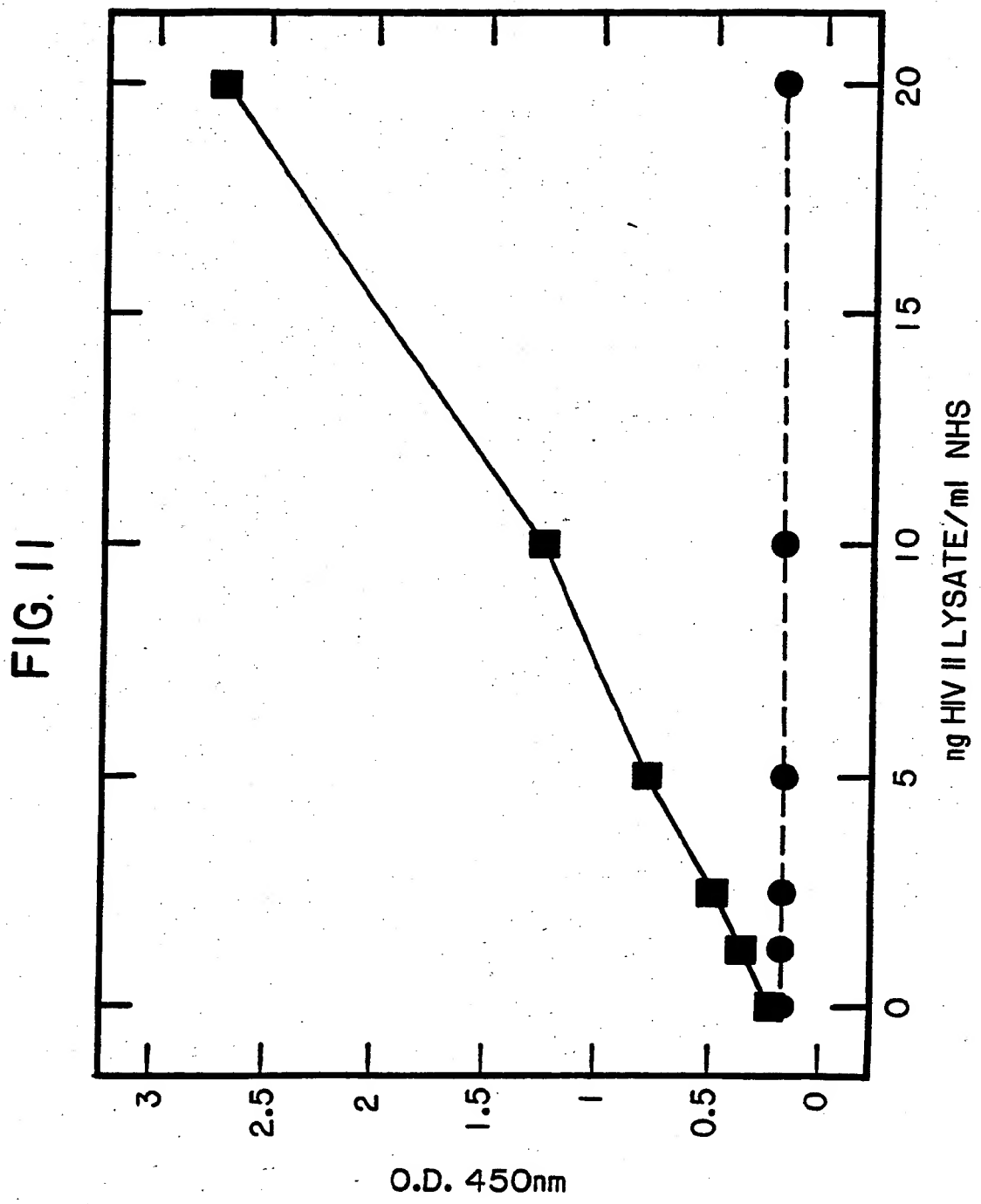


FIG. 11

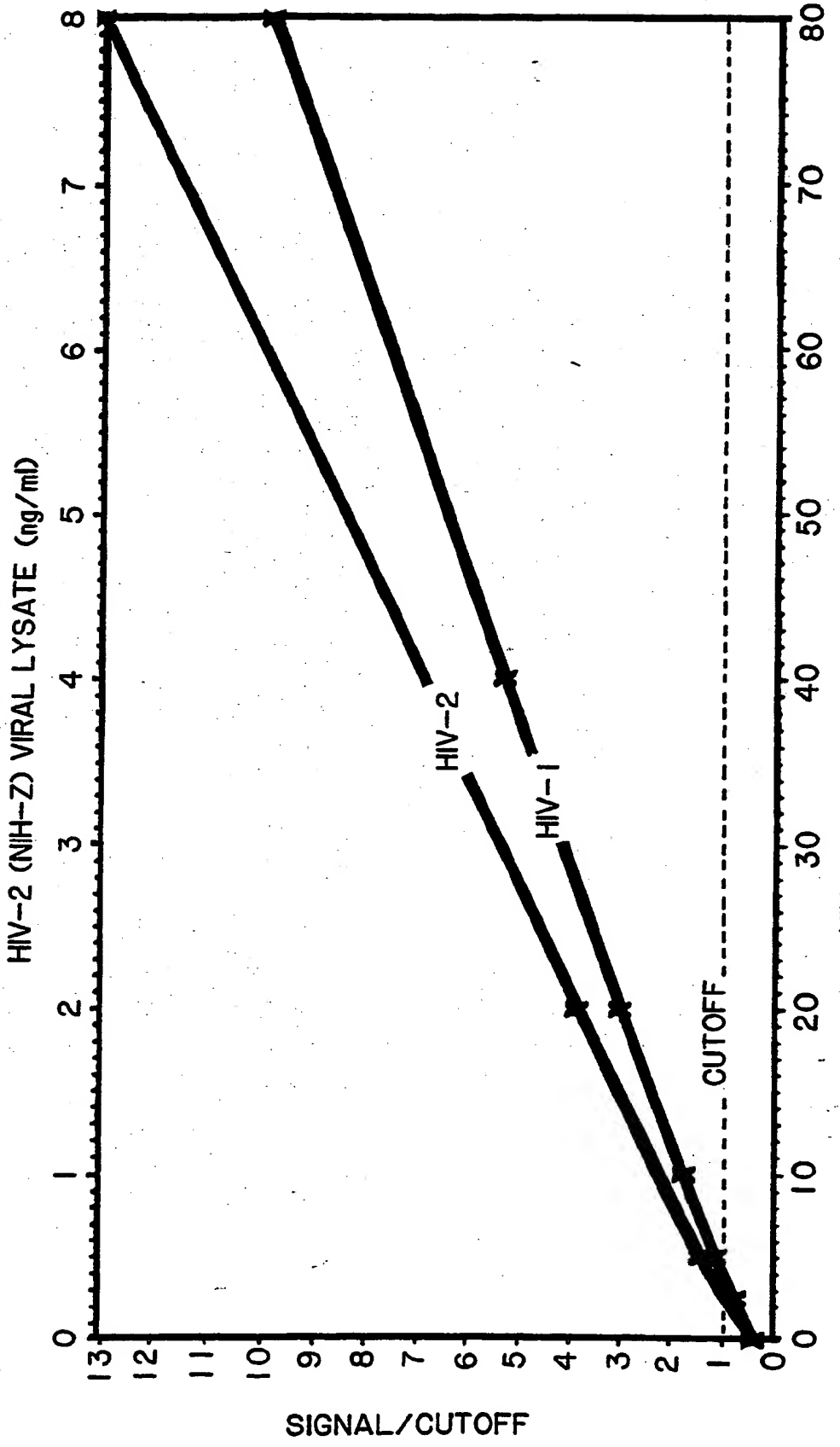
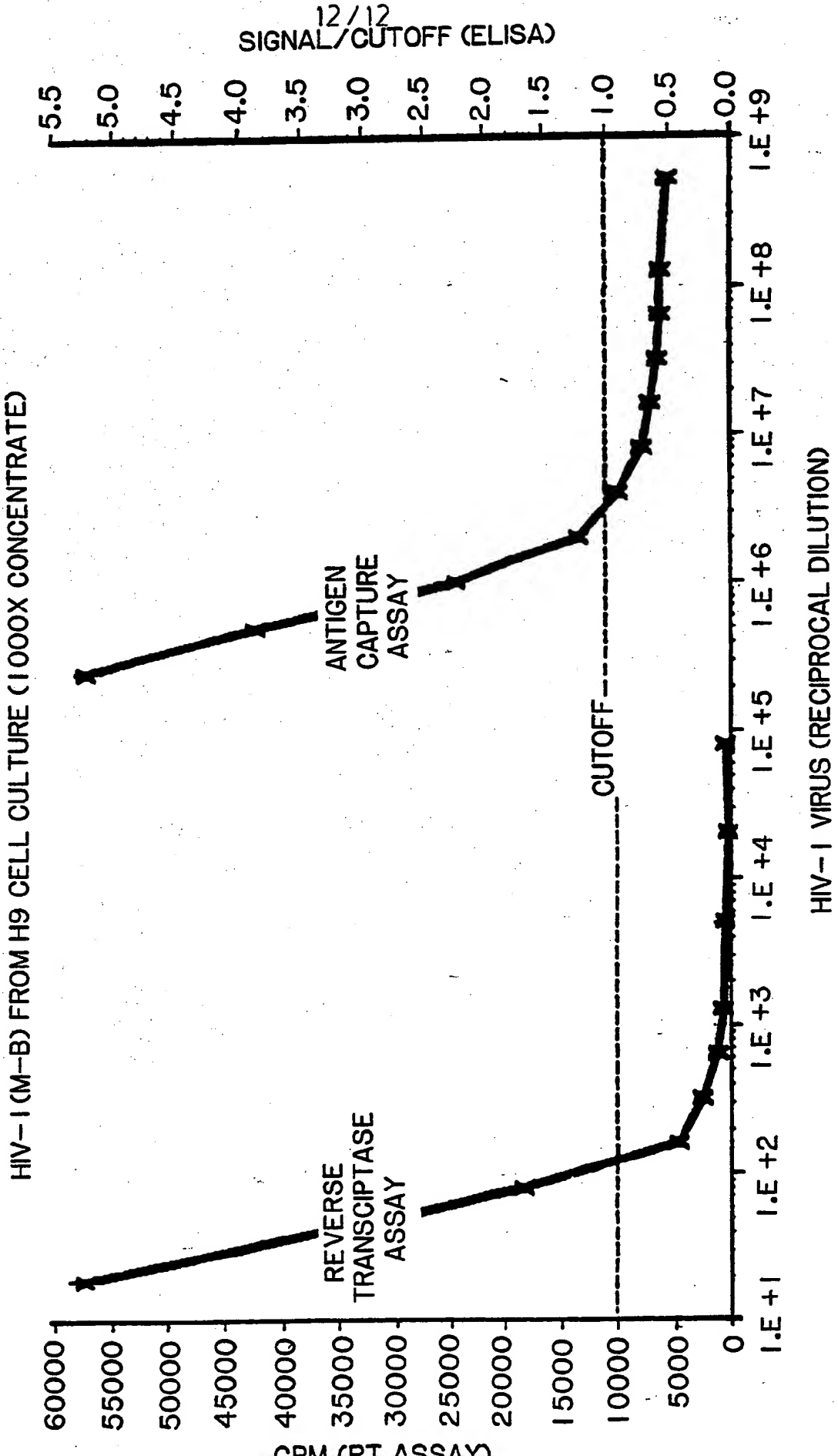

HIV-2 (NIH-Z) VIRAL LYSATE (ng/ml)
HIV-1 (M-B) p24 (pg/ml)
SIGNAL/CUTOFF
HIV-2
HIV-1
CUTOFF
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
13
12
11
10
9
8
7
6
5
4
3
2
1
0

FIG. 12

FIG. 13


SIGNAL/CUTOFF (ELISA)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HIV-1 (M-B) FROM H9 CELL CULTURE (1000X CONCENTRATED)
ANTIGEN CAPTURE ASSAY
CUTOFF
REVERSE TRANSCIPTASE ASSAY
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
CPM (RT ASSAY)
1.E+1
1.E+2
1.E+3
1.E+4
1.E+5
1.E+6
1.E+7
1.E+8
1.E+9
HIV-1 VIRUS (RECIPROCAL DILUTION)

To further localize the epitope of 7-D4, synthetic sequential overlapping nonapeptides were made for the B region of p24. Each nonapeptide served as the solid phase antigen in a series of ELISA's to determine maximal binding affinity of the monoclonal. A single peak of reactivity was found (Figure 7) for a linear domain comprising the region containing amino acids 142-158 (Figure 8).

A comparison of the amino acid sequences of p24 of an HIV-1 isolate, p26 of an HIV-2 isolate and p27 of SIV_{MAC} revealed conservation of a decapeptide (Figure 9) within the epitope of p24 consisting of Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys. It can be inferred that the region encompassing the decapeptide is the 7-D4 epitope of p26 in HIV-2 and p27 in SIV_{MAC}.

The values of a defined epitope are known to those skilled in the art. One of the benefits is the ability of generating new antibodies capable of reacting with said epitope and similar epitopes. Synthetic peptides are configured after the epitope sequence and either unmodified or conjugated to carriers are used as antigen. For example, peptides can be conjugated to PPD, tetanus toxoid, KLH or BSA using glutaraldehyde, carbodiimide or N-maleimidobenzoyl hydroxuccinimide ester. For a review of using synthetic peptides as antigen, see Ciba Foundation Symposium 119 (1986) John Wiley and Sons, NY. Antibodies may be raised in vivo as in mice, goats or other lab animals or in vitro using a system f materials and methods similar to the IVIS of Hana Biologics (Alameda, CA). Another benefit is that large quantities of the epitope s quence can be produced synthetically r using standard recombinant DNA techniques as described above and the p ptides can serve as antigen in immunology-bas d assays and kits f r the det ction of circulating antibody or for the detection

Another benefit relates to improving the assays disclosed herein. Without extending the survey, it is unclear whether the epitope identified in the HIV-1 isolate described herein is specific to that isolate and furthermore to the HIV-2 and SIV isolates described herein. Using that sequence as a reference point, the epitope can be engineered, that is substituting one or more amino acids or alternatively derivitizing the epitope, etc., with a view to identifying a related sequence with a greater degree of conservation among a larger variety of HIV isolates or to obtaining a related sequence with a greater degree of reactivity in assays. Although the nonapeptide analysis apparently identified a discrete linear epitope comprised of amino acids 142-158 of the HIV-1 gag that is conserved in HIV-2 and SIV, it is to be understood that the instant invention relates to monoclonal antibodies, epitopes of said monoclonal antibodies and assays using said antibodies and said peptides that are capable of detecting gag encoded proteins of HIV-1, HIV-2 and SIV.

Capture ELISA Assay

To determine which of the monoclonals would find utility in an ELISA, each was used as a capture or HRP-conjugate antibody in a sandwich assay. Briefly, the monoclonal antibody was coated on wells and 10 µl of disruption buffer added. The antigen samples suspended in detergent buffer or controls in a volume of 100 µl were added next and incubated at 37°C for 90 minutes. After washing, bound antigen was detected by adding to the wells an enzyme conjugated anti-HIV reagent (horseradish peroxidase-conjugated human anti-HIV IgG, affinity purified, 100 µl) and incubated at 37°C for 30 minutes. After washing several times, 100 µl of substrate solution were added to the wells and incubated

at room temperature for 30 minutes. 100 µl of stop reagent were added and absorbance read at 450 nm using an air blank. Representative data are presented in Table 5.

Table 5

Checkerboard Analysis of mAbs

Capture Antibody	5B4	5D9	5E2	6C10	6E11	7E10	9B7	HαHIV
5B4	0.12	0.26	0.29	0.82	0.13	1.03	0.17	2.67
5D9	0.73	0.13	0.43	0.62	0.37	0.38	0.12	>3.0
5E2	0.58	0.47	0.14	0.61	0.23	0.80	0.11	2.51
6C10	0.81	0.38	0.44	0.20	0.17	0.70	0.13	>3.0
6E11	0.09	0.21	0.21	0.14	0.16	0.27	0.09	0.41
7E10	0.84	0.43	0.49	0.84	0.18	0.18	0.13	>3.0
9B7	0.14	0.11	0.10	0.17	0.13	0.17	0.13	0.28
34A	0.49	0.12	0.08	0.96	0.28	1.81	0.22	>3.0

Purified mAb were coated overnight at 10 µg/ml. HRP-mAb used at 10 µg/ml added at beginning of incubation (90′ at 37°C).

HRP-human-anti-HIV was added after 60 min.

Absorbances given for 10.0 ng/ml HIV-1 MOLT 3 in NHS.

Absorbance for NHS was 0.12 ± 0.03

Antibodies 5-B4, 6-C10 and 7-E10 worked best as both capture and conjugated antibodi s. Maximal signals were obtained with the HRP-human anti-HIV as the conjugate.

Vari us combinations of the monoclonals wer used

B4 and 6-C10 showed the greatest sensitivity in detecting p24 (Figure 10). To detect p26 of HIV-2, 7-D4 was used as a capture antibody (Figure 11). It was found that maximal sensitivity and robustness occurred when the three antibodies, 5-B4, 6-C10 and 7-D4 were combined as capture antibodies. Under those conditions, p26 was detectable as well as p24 from certain borderline clinical samples that were difficult to interpret when only 5-B4 and 6-C10 were used as capture antibodies. The sensitivity of the capture ELISA using these three antibodies is less than 10 pg/ml (less than 1 pg/well) of HIV-1 p24 antigen and less than 0.5 ng/ml of HIV-2 p26 antigen (Figure 12). The sensitivity is found despite the presence of HIV antibodies in the clinical samples. A capture ELISA using the three antibodies 5-B4, 6-C10 and 7-D4 was also compared to a reverse transcriptase assay for the detection of whole virus. The ELISA was 25,000 times more sensitive than the reverse transcriptase assay (Figure 13).

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that this disclosure is intended in an illustrative rather than in a limiting s nse, as it is cont mplated that modifications will readily occur to those skilled in the art, within the spirit of the

References

1. Clavel, F., et al., Science 233, 343 (1986)
2. Coates, A., et al., Nature 326, 549 (1987)
3. Guyader, M., et al., Nature 326, 662 (1987)
4. Kessler, H., et al., J Am Med Assoc 258, 1196 (1987)
5. Kohler, G, & Milstein, C., Nature 256, 495 (1975)
6. Kuhnel, H., et al., Proc Natl Acad Sci USA 86, 2383 (1989)
7. Marlink, R., et al., AIDS Res Hum Retroviruses 4, 137 (1988)
8. Minassian, A., et al., Proc Natl Acad Sci USA 85, 6939 (1988)
9. Meissner, P.S. et al., Proc Natl Acad Sci USA 84, 4171 (1987)
10. Niedrig, M., et al., J Gen Virol 69, 2109 (1988)
11. Robert-Guroff, R.C. et al., Science 215, 975 (1982)
12. Sarngadharan, M.G., et al., Science 224, 506 (1984)
13. Starcich, B.R., et al., Cell 45, 637 (1986)
14. Towbin, H. et al., Proc Natl Acad Sci USA 76, 4350 (1979)
15. Wall, R., et al., Lancet i, 566 (1987)
16. Weiss, R., et al., AIDS 2, 95 (1988)

WHAT IS CLAIMED IS:

1. A monoclonal antibody which reacts with an epitope of p24 of HIV-1 and p26 of HIV-2, said epitope located within amino acid residues 140-160 of p24.

2. The monoclonal antibody of claim 1 wherein said epitope is located within amino acid residues 142-158 of p24.

3. The monoclonal antibody of claim 1 wherein said epitope is located within amino acid residues 144-158 of p24.

4. The monoclonal antibody of claim 1 wherein said epitope comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

5. The monoclonal antibody of claim 1 wherein said epitope comprises the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

6. A monoclonal antibody which reacts with an antigen comprising the amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

7. A mon clonal antib dy which reacts with an antig n comprising the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

8. An epitope comprising th amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with

biologic function and with which the monoclonal antibody of claim 1 reacts.

9. An epitope comprising the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys with which the monoclonal antibody of claim 1 reacts.

10. The amino acid sequence His-X-X-X-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-X wherein X is any amino acid compatible with biologic function.

11. The amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

12. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which reacts with an antigen of HIV-1 and a monoclonal antibody of claim 1.

13. The diagnostic kit of claim 12 wherein said antibody is a monoclonal antibody.

14. The diagnostic kit of claim 13 wherein the epitope of said antibody comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

15. The diagnostic kit of claim 14 which contains two monoclonal antibodies which react with an antigen of HIV-1.

16. The diagnostic kit of claim 15 wherein one of said monoclonal antibodies which react with an antigen of HIV-1 binds with an epitope 1 cated within amino acid residues 142-209 of p24 and the second of said monoclonal antibodies which react with an antigen

of HIV-1 binds with an epitope located within amino acid residues 263-344 of p24.

17. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which react with an antigen of HIV-1 and the monoclonal antibody of claim 6.

18. A diagnostic kit for detection of HIV-1 and HIV-2 comprising at least one antibody which react with an antigen of HIV-1 and the monoclonal antibody of claim 7.

19. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 1, and measuring the formation of antigen-antibody complexes.

20. The method of claim 19 wherein said antibody is a monoclonal antibody.

21. The method of claim 20 wherein said epitope comprises the amino acid sequence Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys.

22. The method of claim 21 which contains two monoclonal antibodies which react with an antigen of HIV-1.

23. The method of claim 22 wherein one of said monoclonal antibodies which r act with an antigen of HIV-1 binds with an epitope located within amino acid residues 142-209 of p24 and the second of said monoclonal antibodies which react with an antigen

of HIV-1 binds with an epitope located within amino acid residues 263-344 of p24.

24. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 6, and measuring the formation of antigen-antibody complexes.

25. A method for detection of HIV-1 and HIV-2 antigens in a sample which comprises contacting said sample with at least one antibody which reacts with an antigen of HIV-1 and the monoclonal antibody of claim 7, and measuring the formation of antigen-antibody complexes.

26. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 8 and measuring the formation of antigen-antibody complexes.

27. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the epitope of claim 9 and measuring the formation of antigen-antibody complexes.

28. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the amino acid sequence of claim 10 and measuring the formation of antigen-antibody complexes.

29. A method for detection of HIV-1 and HIV-2 antibodies in a sample which comprises contacting said sample with the amino acid sequence of claim 11 and measuring the formation of antigen-antibody complexes.

30. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the epitope of claim 8.

31. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the epitope of claim 9.

32. A diagnostic kit for detection of HIV-1 and HIV-2 antibodies in a sample comprising the amino acid sequence of claim 10.

33. A diagnostic kit for det ction of HIV-1 and HIV-2 antibodi s in a sample comprising the amino acid sequence of claim 11.